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## HYPOGLYCEMIC, ANTI-INFLAMMATORY AND ANALGESIC ACTIVITY OF *PEPEROMEA PELLUCIDA* (L.) HBK (PIPERACEAE)

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### Keywords:

*Peperomia pellucida*, Hypoglycemic activity, Anti-inflammatory activity, Analgesic activity

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### ABSTRACT

The aspire of the present research was to explore the hypoglycemic, analgesic and anti-inflammatory activity of ethyl acetate extract of *Peperomia pellucida* in alloxan-induced diabetic mice, Acquired immune deficiency syndrome (AIDS), and hypercholesterolemia and against pain. In the present study hypoglycemic effect of the ethyl acetate extract of *Peperomia pellucida* was significant from (25.35 to 8.1 mM  $\pm$ SEM) i.e., 68.44% in 300mg/kg ethyl acetate extract as a 7 days hypoglycemic treatment. On 120 minutes OGTT test, reduction of blood glucose level was also significant. In 300mg/kg extract blood glucose level reduced from (20.53mM to 7.69  $\pm$ SEM) 62.64% was observed. Analgesic treatment was observed carefully and mentionable activities were evaluated. Percentage of inhibition was 58.16 with the number of writhing 11.8 $\pm$ SEM in 300mg/kg inhibition was observed with 6.8  $\pm$ SEM writhing. *P. pellucida* has significant anti-inflammatory effect after 4 hr with 3.47 $\pm$  SEM. The present study indicates significant hypoglycemic, analgesic and anti-inflammatory effects of *P. pellucida*. The present investigation established the pharmacological evidence to support the folklore claim and that of the plant has antidiabetic, analgesic and anti-inflammatory activity.

**INTRODUCTION:** *Peperomia pellucida* (L.) HBK is also known as shiny bush or silver bush belonging to family Piperaceae. In Sanskrit, it is known as Toyakandha, Varshabhoo. *Peperomia pellucida* is an herbaceous plant found in many South American and Asian countries. The species develops during rainy periods (often in the spring) and thrives in loose, humid soils under the shade of trees.<sup>1-4</sup> It grows in moist habitat and is found throughout the major parts of India. In different parts of India it is known with different names like Lochi pata in Bangali<sup>5</sup>, Mashitandu chedi in Malayalam and Pononoa in Assamese etc.<sup>5,6</sup>

Whole plant or parts of plant are used for different purposes. Despite its wide range of folk medicinal uses in India sub-continent, there is very little scientific documentation available on its pharmacological and biological activities as well as its chemical constituents. *Peperomia pellucida* leaves and stems may also be eaten as vegetable<sup>7</sup>. In salads, the fresh plant has the crispness of carrot sticks and celery. As Ethno-medicinal uses of this plant *Peperomia pellucida* has been applied for treating abdominal pain, abscesses, acne, boils, colic, fatigue, gout, headache, renal disorders, and rheumatic joint pain<sup>6,8,9,10</sup>.

In Bolivia, Alenos Indians use the whole plant to stop hemorrhages. The roots are used to treat fevers and the aerial parts are used as dressing for wounds<sup>11</sup>. In northeastern Brazil, the plant has been used to lower the cholesterol level<sup>12</sup>. In Guyana and the Amazon region, it is a popular cough suppressant, emollient, and diuretic. It is also used to treat proteinuria<sup>3,4</sup>. In the Philippines, a decoction of the plant is used to decrease uric acid levels and to treat renal problems<sup>5</sup>.

In different region of Lakshmipur district of Bangladesh, the leaves of the plant are used by local people in the treatment of excited mental disorder<sup>13</sup>. It is also used topically for skin disorders such as acne and boils. In South America, A solution of the fresh juice of stem and leaves is used against eye inflammation<sup>6</sup>. Infusion and decoction of leaves and stems are used for gout and arthritis. According to Manila Medical Society *P. pellucida* is used to relieve arthritic pains, but can cause CNS depression<sup>14</sup>.

This plant has externally used as a facial rinse for complexion problems. Pounded whole plant used as warm poultice for boils, pustules and pimples and also used for headaches, rheumatic pains and impotence<sup>15</sup>. *Peperomia pellucida* is also used in traditional Ayurvedic medicine<sup>6</sup>. It is described in Ayurveda as – Rasa – Katu and Madhur; Guna- Lakhu, rooksha, Teekshna; and Virya- Ushna. The plant is described to passify vitiated cough, pitta, constipation, kidney diseases, urinary retention, dysuria, urinary tract infections, emaciation, edema and general weakness. Infusion and decoction of leaves and stems of fresh plant are eaten as salad for the treatment of gout and arthritis<sup>15,16</sup>.

According to Ethno-botanical studies the whole plant has been in medicinal use since long. It is crushed and mixed with water to form a mixture, heated and administered orally to cure hemorrhage. It is also been applied against coughing, fever, common cold, headache, sore throat, diarrhea, against kidney and prostate problems and against high blood pressure<sup>17</sup>. The analgesic properties of the plant seem to be related to its effect on prostaglandin synthesis<sup>18</sup> it may have potential as a broad spectrum antibiotic, as demonstrated in tests against *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Escherichia coli*<sup>19</sup>.

Chloroform extracts from dried leaves of *P. pellucida* have been shown to exhibit antifungal activity against *Trichophyton menta- grophytes in vitro*<sup>20</sup>. Although the plant can cause asthma-like symptoms in patients with known hypersensitivity reactions to the species, no clinical data have yet been reported on human toxicity.

## MATERIALS AND METHODS:

**Plant Materials:** *Peperomia pellucida* were collected from the side of the river in Fakirhat, Khulna, Bangladesh during the month of October in 2011 and the plant authenticity were confirmed from the Bangladesh National Herbarium, Mirpur, Dhaka.

**Preparation of Plant Extracts:** The whole plant of *Peperomia pellucida* were dried in an oven at 37°C and then powdered with a mechanical grinder, passing through sieve #40 and stored in a tight container. The dried powdered material (1.5kg) was dissolved with methanol for 5 days. The total filtrate was concentrated to dryness, to render the methanol extract (490 g). This extract was then suspended in water and then successively partitioned with ethyl acetate (EtOAc) and Chloroform (CHCl<sub>3</sub>) fraction along with the residue present in aqueous phase.

**Drugs and Chemicals:** The standard drug, Metformin hydrochloride was the generous gift samples from Beximco Pharmaceuticals Ltd of Bangladesh. Alloxan monohydrate was purchased from Loba chemie, India. Carrageenan was purchased from Otta chemika, India. Blood samples analyzed for blood glucose content by using OK meter Match glucose test meter (Hsinchu, Taiwan). Acetic acid was collected from laboratory of Bangladesh University. The standard drug Diclofenac-Na was purchased from Square Pharmaceuticals Limited of Bangladesh.

**Experimental Animals:** Eight week-old male Swiss albino mice (27-30g) purchased from Jahangirnagar University, Dhaka Bangladesh and were housed in animals cages under standard environmental conditions (22-25°C, humidity 60-70%, 12 h light: 12 h dark cycle). The mice were feed with standard pellet diet taken from Jahangirnagar University, Dhaka and water *ad libitum*. The animals used in this study were cared in accordance with the guidelines on animal experimentation of our institute.

**RESULTS AND DISCUSSIONS:**

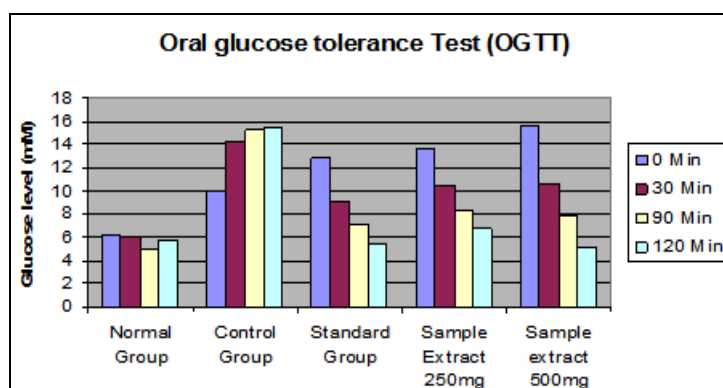
**Oral Glucose Tolerance Test (OGTT) of *Peperomia pellucida* extract in Alloxan induced Diabetic mice:**

After oral administration of glucose the blood glucose levels were significantly higher in diabetic and experimental groups of mice as shown in **Table 1** and

**TABLE 1: EFFECT OF THE ETHYL ACETATE EXTRACT OF *PEPEROMIA PELLUCIDA* (LEAF AND STEM) ON ORAL GLUCOSE TOLERANCE TEST IN DIABETIC MICE**

Group	0 min	30 min	90 min	120 min
Normal group	6.27±0.86	5.97±0.58	5.1±1.058	5.77±0.451
Group DC	9.97±1.56	14.10±1.21	15.23±0.81	15.43±0.814
Group DS	12.83±2.51	9.1±0.458	7.13±0.404	5.37±0.8504
Group DPP (300mg/kg)	13.53±1.95	10.43±0.472	8.267±1.0	7.67±0.55

Values were expressed in Mean ± Standard Error. Each group comprised 5 animals. Control group received 0.5% Methyl cellulose and standard group received 120mg/kg Metformin.

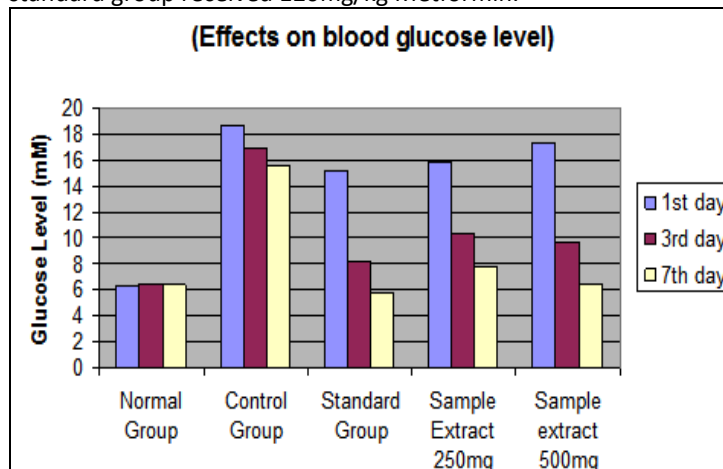


**FIG. 1: EFFECT OF THE ETHANOLIC EXTRACT OF *PEPEROMIA PELLUCIDA* (LEAF AND STEM) ON ORAL GLUCOSE TOLERANCE TEST IN DIABETIC MICE**

**TABLE 2: HYPOGLYCEMIC EFFECT OF THE ETHYL ACETATE EXTRACT OF *PEPEROMIA PELLUCIDA* (LEAF AND STEM) ON DIABETIC MICE**

Groups	0day	1st day	3rd day	7th day
Normal group	6.27±0.86	5.97±0.58	5.1±1.058	5.77±0.451
Diabetic Control (DC)	29.5±1.4	26.5±0.70	25.8±2.12	7.5±1.5
Standard (DS)	27.75±1.35	23.3±1.7	10.3±1.4	6.9±1.1
Cassia Fruit (DCF)	25.35±0.69	19.2±2.2	13.3±2.1	8.1±1.5

Values were expressed in Mean ± Standard Error. Each group comprised 5 animals. Control group received 0.5% Methyl cellulose and standard group received 120mg/kg Metformin.



**FIG. 2: HYPOGLYCEMIC EFFECT OF THE ETHANOLIC EXTRACT OF *PEPEROMIA PELLUCIDA* (LEAF AND STEM) ON DIABETIC MICE**

**Fig. 1.** In diabetic control the peak increase in blood glucose concentration was observed after 30 min and remained high over the next hour. Mice treated with extracts in Group-DPP (500mg/kg) showed a significant decrease in blood glucose concentration at 90min and 120min compared with diabetic control mice.

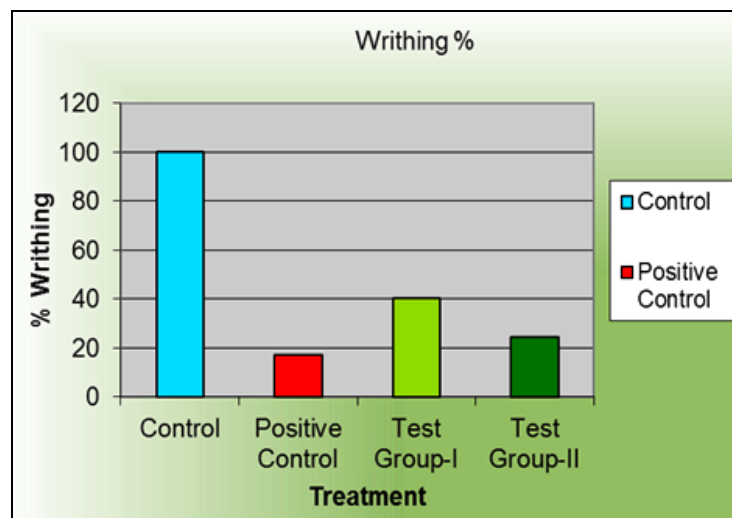
**Hypoglycemic effect of ethyl acetate extract of *Peperomia pellucida* extract in Alloxan induced Diabetic mice:** Table 2 shows antihyperglycemic effect is a drug dependent response (Fig. 2) when performed at the end of the experiment. Hypoglycemic test when performed with the extract showed more improvement compared with that without the extract (DC group). After 7 days of treatment with extracts, glucose levels were significantly lowered in Group-DPP (300mg/kg).

**Analgesic effect of *Peperomia pellucida* extract on acetic acid induced writhing in mice:** Table 3 shows the effects of the extract of an acetic acid-induced writhing in mice. The oral administration of both doses of *Peperomia pellucida* extract significantly ( $p < 0.001$ ) inhibited writhing response induced by acetic acid in a dose dependent manner compared. The writhing count is positive of test group-I and test group-II (Fig. 3) compared to control group. The analgesic effect or percent of pain inhibition effect of extract is near the standard group (Fig. 4). The effect was dose dependent and the most significant effect observed with test group-II (500 mg/kg) which is very close to the standard group compared to control group.

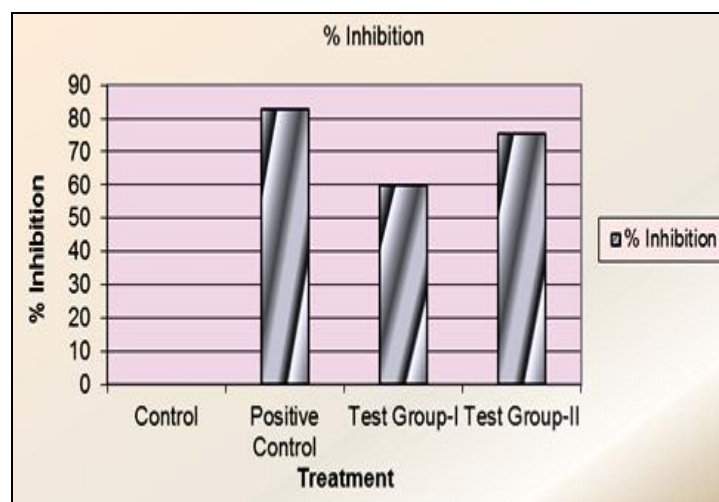
**TABLE 3: EFFECTS OF THE ETHYL ACETATE EXTRACTS OF STEM AND LEAF OF *PEPEROMIA PELLUCIDA* ON ACETIC ACID-INDUCED WRITHING IN MICE.**

Animal Group/ Treatment	Writhing Counting (Mean ± SEM) (% Writhing)	Inhibition (%)
Group-I (Control group) 1% Tween 80 solution in water (10 ml/ kg; P.O.)	33.80 ±0.748 (100)	–
Group-II (Standard group) Diclofenac (10 mg/kg;P.O.)	5.80 ±1.008 (17.16)	82..84
Group-III Ethyl acetate extract (300 mg/kg;P.O)	13.60±0.765 (40.23)	59.77

Values are mean ± SEM, (n=5); \*\*:p<0.001, Dunet test as compared to control group. Group I animal received vehicle (1% Tween 80 in water), Group II received Diclofenac 10 mg/ kg body weight, Group III and Group IV were treated with 250 and 500mg/kg body weight (p.o.) of the crude extract of *Peperomia pellucida*.



**FIG. 3: EFFECTS OF THE ETHANOLIC EXTRACTS OF STEM AND LEAF OF *PEPEROMIA PELLUCIDA* ON ACETIC ACID-INDUCED WRITHING IN MICE**



**FIG. 4: PERCENT OF INHIBITION EFFECTS OF THE ETHANOLIC EXTRACTS OF STEM AND LEAF OF *PEPEROMIA PELLUCIDA* ON ACETIC ACID-INDUCED WRITHING IN MICE.**

**Phytochemical Screening:** Table 4 shows the results of the phytochemical screening of the stem and leaf of *Peperomia pellucida*.

**TABLE 4: PHYTOCHEMICAL SCREENING**

Secondary Metabolites	Name of the Test	Stem & leaf
Alkaloids	Hager's test	+
	Wagner's test	+
Flavonoids	-	++
glycosides	-	++
Saponins	-	+
Carbohydrates	-	+
Tannins	-	-

(+) indicates present and (-) indicates absent

From the above table, (++) sign indicated larger amount of flavonoids and glycosides present in the study sample than others and tannins test was negative.

**DISCUSSION:** Diabetes mellitus is one of the most common chronic disease and is associated with hyperglycemia, polyurea, polydipsia, polyphagia, weight loss, muscle weakness, hyperlipidemia and comorbidities such as obesity, hypertension. Hyperglycemia and Hyperlipidemia are the two metabolic complications of both clinical and experimental diabetes<sup>21</sup>. Alloxan, a β-cytotoxin, induces "chemical diabetes" (alloxan diabetes) in a wide variety of animal species by damaging the insulin secreting pancreatic β-cell, resulting in a decrease in endogenous insulin release, which paves the ways for the decreased utilization of glucose by the tissues<sup>22</sup>.

Apart from the regulation of carbohydrate metabolism, insulin also plays an important role in the metabolism of lipids. Insulin is a potent inhibitor of lipolysis. Since it inhibits the activity of the hormone sensitive lipases in adipose tissue and suppresses the release of free fatty acids. During diabetes, enhanced activity of this enzyme increases lipolysis and releases more free fatty acids into the circulation.

Increased fatty acids concentration also increases the  $\beta$ -oxidation of fatty acids, thus increasing cholesterol level during diabetes. In normal condition, insulin increases the receptor-mediated removal of LDL-cholesterol and decreased activity of insulin during diabetes causes hypercholesterolemia. Hypercholesterolemia and hypertriglyceridemia have been reported to occur in diabetic rats<sup>23</sup>.

In the light of the literature on *Peperomia pellucida*, we made an attempt for the first time to study the effect of *Peperomia pellucida* extract in hyperglycemic mice. The experiment showed that, the extracts have the properties to stimulate or regenerate the  $\beta$ -cell for the secretion of insulin and are most effective for controlling diabetes by various mechanisms due to presence of hypoglycemic alkaloids, saponin and flavonoids.

Oral Glucose Tolerance Test (OGTT) measures the body ability to use glucose, the body's main source of energy<sup>24</sup>. It can be used to diagnose prediabetes and diabetes. In our study, it is found that various fractions have also hypoglycemic effect in glucose induced hyperglycemic mice. The effects of extracts on blood sugar levels are dose dependent. This may be due to the presence of hypoglycemic flavonoids, triterpenes or saponin glycosides that also requiring further investigation.

Hypercholesterolemia and hypertriglyceridemia have been reported to occur in diabetic mice<sup>25-27</sup>. Intraperitoneal administration of ethanolic extracts of *Peperomia pellucida* resulted in significant reduction of serum lipid levels in mice with hyperlipidemia viz. triglyceride and total cholesterol. Flavonoids are known for their diverse activities including hypolipidemic activity resulting from their antioxidant activity<sup>28</sup>.

*Peperomia pellucida* extracts contain the presence of flavonoids and related phenolic compounds. For this lipid lowering capacity of this plant extracts, it may be proposed that the constituents of the plant extracts may act as inhibitors for enzyme such as hydroxy-methyl-glutaryl-CoA reductase, which participates in de novo cholesterol biosynthesis<sup>29</sup>. Induction of diabetes with alloxan was associated with decrease in hepatic glycogen, which could be attributed to the decrease in

the availability of the active form of enzyme glycogen synthetase probably because of low levels of insulin<sup>30, 31</sup>.

In the present study, *Peperomia pellucida* restored the depressed hepatic glycogen levels possibly by increasing the level of insulin. Our result showed that supplementation of diabetic mice with plant extracts resulted in significant elevation in hepatic glycogen content. Decreased activities of the enzymes involved in glucose homeostasis in liver and kidney such as hexokinase has been reported in diabetic animal resulting in depletion of liver and muscle glycogen content<sup>32</sup>. With plant extracts might increase the level of enzyme to the control level indicating an over-all increase in glucose influx. The exact mechanism of action needs further investigation.

Acetic acid-induced writhing model represent pain sensation by triggering localized inflammatory response. Such pain stimulus leads to the release of free arachidonic acid from phospholipids<sup>33</sup>. The acetic acid-induced writhing model response is a sensitive procedure to evaluate peripherally acting analgesic. The response is thought to be mediated by peritoneal mast cells, acid sensing ion channels and the prostaglandin pathway<sup>34, 35</sup>. Preliminary photochemical screening reveals the presence of flavonoid, steroid, alkaloid, tannin and saponin in the plant extract. So the observed analgesic activity may be attributed to these compound.

**CONCLUSION:** The research work was undertaken to evaluate the hypoglycemic properties of different parts of the plant *Peperomia pellucida* in alloxan-induced diabetic mice, analgesic and also anti-inflammatory effects. The most widely used experimental procedures were to examine the effects of plant extracts on blood glucose level both in normal and alloxan-induced diabetic mice, to determine the effects of plant extracts, the analgesic activity for its central and peripheral pharmacological actions using tail immersion and acetic acid induced writhing test in mice. Finally, to find out the possible mechanism action of the plant extracts for their beneficial effect both in normal and alloxan-induced diabetic mice and the present study was designed to evaluate anti-inflammatory activity to the plant extracts of the *Peperomia pellucida* stem and leaf of the plants.

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